SAARC J. Agric., 19(1): 15-25 (2021) *DOI: https://doi.org/10.3329/sja.v19i1.54775*

ASSESSMENT OF GENETIC DIVERSITY OF 62 F_4 POPULATION OF RAPESEED (*Brassica napus* L.) THROUGH MULTIVARIATE ANALYSIS

S. Sultana[1*], F. Mahmud[2] and M. Asaduzzaman[1]

[1]Department of Biochemistry, [2]Department of Genetics and Plant Breeding

Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

ABSTRACT

Rapeseed (*Brassica napus* L.) is the best one in respect of oil production. It is the order of the day to take better steps for production and quality improvement of our local cultivars. Broadening of genetic diversity in spring oilseed *Brassica napus* L. (AACC, 2*n* = 38), canola is important for continued improvement of this crop. Sixty-two genotypes in F_4 generation of *Brassica napus* L. were evaluated to assess genetic diversity based on randomized complete block design with three replications at the experimental field of Sher-e-Bangla Agricultural University, Dhaka. Different Multivariate analyses were performed to classify 62 genotypes. On the basis of cluster analysis, all the genotypes were classified in five clusters. The cluster IV comprised the maximum number (19) of genotypes followed by same in cluster II (18). The cluster I and V comprised 10 and 9 genotypes respectively. The lowest number of genotypes was present in cluster II. The highest inter-cluster distance (10.309) was observed between the cluster I and IV and the genotypes of these clusters involved in hybridization may produce a wide spectrum of segregating population. The lowest inter-cluster distance (3.513) was observed between the cluster III and IV. The inter-cluster distances were larger than the intra-cluster distances. Considering cluster distance, inter genotypic distance and other agronomic performance G3, G4, G24, G35 and G51 might be suggested for future breeding program.

Keywords: Cluster analysis, Genetic diversity, Principal component analysis, Rapeseed.

[*] Corresponding author: sau.sharmin007@gmail.com

Received: 17.02.2021 *Accepted: 29.05.2021*

INTRODUCTION

The genus *Brassica* L. holds the most economically valuable position in the tribe Brassiceae, which is a part of family Brassicaceae which comprises of 338 genera and 3709 species. (Bilal et al., 2015). The genus is remarkable for containing more important agricultural and horticultural crops than any other genus Most are annual or biennial, but some are small shrubs. *Brassica napus* L. also known as rapeseed, oilseed rape and canola, is the best one with respect of oil production. Rapeseed originated in either the Mediterranean area or Northern Europe. *Brassica napus L.* is the second most important oilseed crop in the international oilseed market following soybean (Sharafi et al., 2015). According to FAO (2019) During 2019, rapeseed/ mustard was globally grown on area of approximately 34.5 million ha with the total production 70 million metric tons. Rapeseed has a wide range of applications such as cooking oil, element of beautifying agents but also pesticides, biofuels, etc. It is the third-largest used source of vegetable oil in the world (USDA, 2002).

It is the most important oilseed crop in Bangladesh and it occupies the 1st position in respect of area and production among the oil crops grown in Bangladesh (BBS, 2019). *Brassica* is grown throughout the country as a single or in combination with other crops like wheat, chickpea, etc. in both irrigated and non-irrigated regions of the country. In Bangladesh, 667242 acres of land was under rapeseed cultivation during 2018-19 which produced about 311740 metric tons of seed (BBS, 2019). Bangladesh has been facing acute shortage of edible oil for the last several decades. Total consumption of oils and fats was 3.04 million tons in 2019 and import edible oil which cost 1161 million US$ (BER, 2019). The major reasons for such poor yield in Bangladesh may be attributed due to pressure of other crops, lack of improved varieties and poor management practices.

In spite of the large benefits and as a good source of vegetable oil it is used in minute amounts because of very high amount of erucic acid and glucosinolates which is harmful for the cardiac muscle and makes the animal feed weaker and innutritious (O'Brien, 2008). There are important different breeding strategies such as understanding and utilization of genetic resources, physiological and morphological basis of yield linked traits in different environmental conditions for the improvement of seeds yield and adaptability of rapeseed and other *Brassica* species. In that respect so many strategies are applied for the enhancement of quality and yield of different canola varieties and cultivars to gain handsome production. Due to application of different techniques such as microspore culture for the production of doubled-haploid lines, wide hybridizations using embryo rescue techniques, or protoplast fusion are involved in creating novel genetic variation, marker-assisted

selection and genetic engineering in breeding process remarkable improvement has been brought in both productivity and quality of canola oil for using it in human diet.

Genetic diversity is the base of improvement, if there were no diversity in nature no crop improvement would possible. Genetic diversity serves as a way for populations to adapt various environments. It arises either due to geographical separation or due to genetic barriers to cross ability. It plays an important role in plant breeding because hybrids between lines of diverse origin generally display a great heterosis than those between closely related strains (Singh, 1983) which permits to select the genetically divergent parents to obtain the desirable recombination in the segregating generations. The present study was, therefore, undertaken to analyze the genetic divergence among 62 genotypes of *Brassica napus* L. in F_4 generation.

MATERIALS AND METHOD

A field experiment was carried out in the experimental fields of Sher-e-Bangla Agricultural University, Dhaka during November 2015 to February 2016. The healthy seeds of 62 genotypes of *Brassica napus* L. in F_4 generation (Table 1), which were collected from the Dept. of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University. Parent plants were Nap- 9908, Nap- 179, Nap- 2001, Nap- 248, Nap- 159, Nap- 2037, Nap- 2057, Nap- 94006, Nap- 2012, Nap- 2013, Nap- 206, Nap- 2022, Bs- 13, Bs- 7 and 62 crosses among them. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The total area of the experiment was $56m \times 14m = 784m^2$. Each replication size was $56m \times 3.5m$, and the distance between replication to replication was 1m. The spacing between lines to line was 30 cm.

Data were recorded on selected characters viz., plant height (cm), no. of primary branches/plant, no. of secondary branches/plant, days to 50% flowering, days to maturity, siliquae/plant, siliqua length, seeds/siliqua, 1000-seed weight and yield/plant from randomly selected 15 plants. Genetic diversity was worked out following Mahalanobis generalized distance (D2) extended by Rao (1952). Clustering of genotypes was done according to Tocher's method (Rao, 1952) and (Singh, 1985). All the statistical analyses were done using GENSTAT 5.13 softwares program.

Table 1. List of genotypes used in this study

Genotype ID	F4 Population	Genotype ID	F4 Population
G1	Nap- 9908 × Bs- 13	G32	Nap- 248 × Nap- 2013
G2	Nap- 179 × Nap- 2001	G33	Nap- 179 × Nap- 2057
G3	Nap- 248 × Nap- 159	G34	Nap- 179 × Nap- 2022
G4	Nap- 2037 × Nap- 2057	G35	Nap- 2037 × Nap- 2013
G5	Nap- 94006 × Bs- 7	G36	Nap- 248 × Nap- 2057
G6	Nap- 2012 × Nap- 2013	G37	Nap- 94006 × Nap- 2057
G7	Nap- 94006 × Nap- 2013	G38	Bs- 7 × Nap- 2013
G8	Nap- 248 × Nap- 206	G39	Nap- 2057 × Nap- 2001
G9	Nap- 206 × Nap- 2012	G40	Bs- 13 × Nap- 2001
G10	Nap- 2037 × Nap- 2022	G41	Nap- 94006 × Nap- 2001
G11	Nap- 9908 × Nap- 94006	G42	Bs- 13 × Nap- 2057
G12	Nap- 9908 × Nap- 2037	G43	Nap- 179 × Nap- 2012
G13	Nap- 2037 × Nap- 248	G44	Nap- 2001 × Nap- 179
G14	Nap- 206 × Nap- 2013	G45	BS- 13 × Nap- 179
G15	Bs- 7 × Nap- 206	G46	BS- 7 × Nap- 2057
G16	Nap- 2001 × Nap- 2022	G47	Nap- 206 × Nap- 2022
G17	Nap- 94006 × Bs- 13	G48	Nap- 206 × Nap- 2057
G18	Nap- 2037 × Nap- 2012	G49	Nap- 9908 × Nap- 2012
G19	Nap- 2037 × Nap- 206	G50	Nap- 179 × Nap- 2013
G20	Nap- 9908 × Nap- 2022	G51	Nap- 248 × Nap- 2012
G21	Bs- 13 × Nap- 2022	G52	Nap- 2057 × Nap- 248
G22	Nap- 179 × Nap- 206	G53	BS- 7 × Nap- 2013
G23	Nap- 9908 × Nap- 206	G54	Nap- 94006 × Nap- 179
G24	Nap- 9908 × Nap- 248	G55	Nap- 2001 × Nap- 2013
G25	Nap- 2012 × Nap- 2022	G56	Nap- 94006 × Nap- 2022
G26	Nap- 248 × Nap- 2022	G57	Nap- 2057 × Nap- 2012
G27	Bs- 13 × Nap- 2013	G58	Nap- 2001 × Nap- 248
G28	Nap- 9908 × Nap- 2001	G59	Nap- 2057 × Nap- 2022
G29	Nap- 2037 × Bs- 13	G60	Nap- 94006 × Nap- 2012
G30	Bs- 13× Nap- 206	G61	Nap- 94006× Nap- 206
G31	Nap- 9908 × Nap- 2013	G62	Nap- 2001 × Nap- 206

RESULTS AND DISCUSSION

Genetic diversity is the total number of genetic characteristics in the genetic makeup of a species. But during continuous selection process for better quality and

productivity, the gene pool of the selected final varieties has been made narrow down due to eliminating of genes for undesirable traits like, declining amount of erucic acid in oil and glucosinolates in seeds. The genetic divergence and clustering of the genotypes were studied based on the characters studied and presented in below.

Principal component analysis (PCA)

The analysis of variance showed significant differences among the genotypes for all the 10 characters under this study revealing the presence of notable genetic variability among the genotypes. The principal component analysis (PCA) showed eigen values and percent of variation in respect of ten component characters of sixty-two F_4 genotypes of *Brassica napus* L. genotypes of (Table 2). The PCA gives Eigen values of principal component axes of coordination of genotypes with the first axes totally accounted for the variation among the genotypes (40.44). These three principal components account for 77.3% of the total variation (Table 2). Zaman et al. (2010) reported that first three axes accounted for 94.00% of the total variation whereas the first principal components accounted for 81.94%. Khan (2014) reported that the contribution of first three PCs in overall PCs was 26.96%.

Table 2. Eigen values and yield percent contribution of 10 characters of 62 F_4 genotypes of *Brassica napus* L.

Principal component axes	Eigen values	Percent variation	Cumulative % of Percent variation
I	4.044	40.44	40.44
II	1.994	19.94	60.38
III	1.692	16.92	77.3
IV	0.836	8.36	85.66
V	0.622	6.22	91.88
VI	0.385	3.85	95.73
VII	0.192	1.92	97.65
VIII	0.135	1.35	99
IX	0.057	0.57	99.57
X	0.043	0.43	100

A two dimentional scatter diagram was constructed using components I and II as the axes (Figure 1). The genotypes were apparently distributed into five clusters (Figure 2). The genotypes were distantly located from each other. The genotypes of cluster I was more diverse than those of cluster III (Figure 2). Begum et al. (2007) reported five clusters and Rameeh (2013) reported 4 clusters in rapeseed.

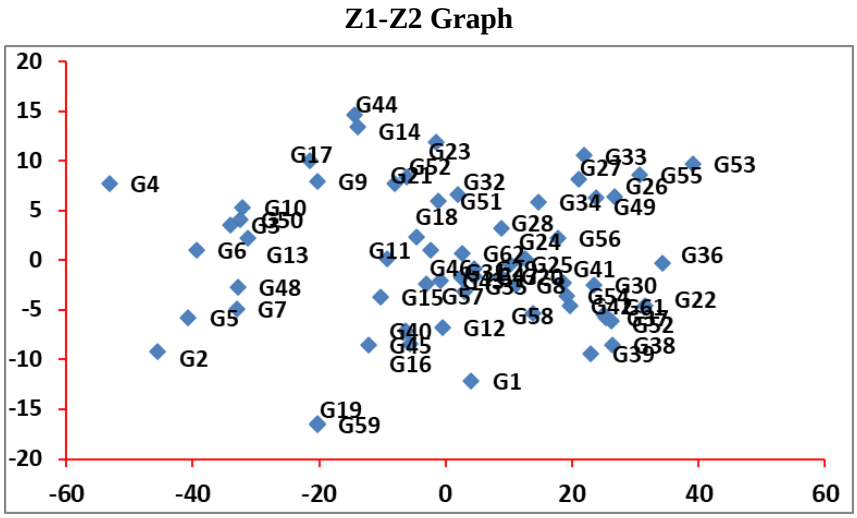

Figure 1. Scatter pattern of *Brassica napus* genotypes of based on their principal component scores

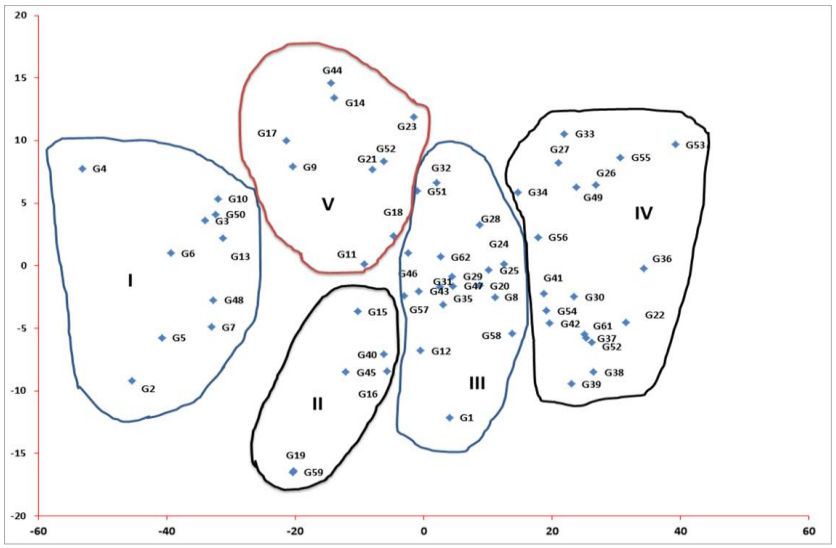

Figure 2. Scatter diagram of *Brassica napus* genotypes of based on their principal component scores.

Nonhierarchical clustering

Non-hierarchical clustering using covariance matrix among 62 genotypes of rapeseed grouped them into five clusters. (Table 3). Maximum number of genotypes (19) was

comprised into cluster IV followed by 18 in cluster III. The cluster I has 10 genotypes followed by 9 genotypes in cluster V. The minimum genotypes (6) was groped into cluster II (Table 3). Rameeh (2015) reported three clusters in 21 rapeseeds genotypes which were selected based on diversity of agronomic characters.

Table 3. Distribution of genotypes in different clusters

Cluster no.	ID of Genotypes	No. genotypes
I	G2, G3, G4, G5, G6, G7, G10, G13, G48, G50	10
II	G15, G16, G19, G40, G45, G59	6
III	G1, G8, G12, G20, G24, G25, G28, G29, G31, G32, G35, G43, G46, G47, G51, G57, G58, G62	18
IV	G22, G26, G27, G30, G33, G34, G36, G37, G38, G39, G41, G42, G49, G53, G54, G55, G56, G60, G61	19
V	G9, G11, G14, G17, G18, G21. G23, G44, G52	9
	Total	62

Cluster mean analysis

The cluster mean values for the 10 characters are given in Table 4. The genotypes from cluster I earned the highest cluster mean value for number of primary branches per plant (3.30), number of secondary branches per plant (3.29), number of siliqua per plant (123.65), thousand seed weight (3.52 g) and seed yield per plant (8.75) (Table 4). Thus, it indicates genotype of this cluster could be used for parent in future hybridization program for higher seed yield. On the other hands Cluster II produced the highest mean for seeds per siliqua per plant (21.81) and 1000-seed weight (3.52 g) and lowest plant height (94.71) (Table 4). It is indicated that the genotypes of this cluster could be used for future hybridization program for higher seeds per siliqua. The genotypes included in cluster III were highest mean value for siliqua length (7.78 cm) and lowest mean value for days to 50% flowering (35.41), days to maturity (80.93) and 1000 seed weight (3.27). It is indicated that the genotype of this cluster could be used for future hybridization program for early maturity. Moreover, Cluster IV had lower cluster mean for number of primary branches per plant (2.10), number of secondary branches per plant (1.38), number of siliqua per plant (63.39), siliqua length (7.42), seeds per siliqua (19.61) and seed yield per plant (4.20). On the other hand, cluster V showed the late 50% flowering (39.89), late maturity plant (84.85) and highest plant height (112.55) (Table 4). It indicated the genotype of this cluster could be used for future hybridization program for late maturity plant. Zaman et al. (2010) reported that the highest cluster means for primary branches per plant and maximum seeds per siliquae with minimum seed yield per plant were obtained in cluster II from eighteen advanced lines of mustard.

Table 4. Cluster mean values of 10 different characters of 62 genotypes of *Brassica napus* L.

Characters	I	II	III	IV	V
Days to 50% flowering	35.90	36.17	35.41	35.54	39.89
Days to maturity	82.07	81.56	80.93	81.40	84.85
Plant height (cm)	112.00	94.71	101.37	98.16	112.55
Number of primary branches per plant	3.30	2.95	2.70	2.10	2.89
Number of secondary branches per plant	3.29	2.05	1.88	1.38	2.50
Siliqua per plant	123.65	101.99	83.23	63.39	96.52
Siliquae length (cm)	7.75	7.64	7.78	7.42	7.54
Seeds per siliqua	21.75	21.81	21.64	19.61	20.75
1000-seed weight (g)	3.52	3.52	3.27	3.40	3.50
Seed yield per plant (g)	8.75	6.96	5.74	4.20	6.26

Inter and intra cluster distance

Canonical Variate Analysis (CVA) was done to compute the inter-cluster distances. In this experiment, the inter-cluster distances were higher than the intra-cluster distances thus indicating broader genetic diversity among the genotypes of different groups. Islam and Islam (2000) reported that the inter-cluster distances were larger than the intra-cluster distances in 42 cultivars of mustard. The highest inter-cluster distance was observed between clusters I and IV (10.309), followed by between cluster III and I (7.112), V and IV (6.390), II and IV (6.373) (Table 5). The higher inter-cluster distances between these clusters indicate to obtain wide spectrum variability of population. However, the highest inter cluster distance was observed between clusters I and IV indicated that the genotypes in these clusters were more diverse compared to other clusters as the greater the distance between two clusters the greater the divergence.

The intra cluster distance was highest in cluster IV (0.086) and lowest in cluster II (0.032) (Table 5). The intra-cluster distances in all the five clusters were lower than the inter -cluster distances and which indicated that genotypes within the same cluster were closely related. It is assumed that the maximum amount of heterosis will be manifested in cross combination involving the genotypes belonging to most divergent clusters. Furthermore, for a practical plant breeder, the objective is to achieve high-level production in addition to high heterosis. Pandey et al. (2013) found maximum inter-cluster distance was found between cluster II and III indicating high genetic divergence among genotypes of these groups.

Table 5. Intra (Bold) and inter cluster distances (D^2) for 62 genotypes of *Brassica napus* L.

Cluster	I	II	III	IV	V
I	0.062	5.440	7.112	10.309	4.462
II		0.032	3.605	6.373	4.243
III			0.073	3.513	3.675
IV				0.086	6.390
V					0.047

Contribution of characters towards divergence

Principal component analysis, one of the multivariate techniques, is used to examine the interrelationship among several characters and can be done from the sum of squares and product matrix for the characters. The characters contributing the most to the divergence are given greater importance when deciding on the cluster for the purpose of further selection and choice of parents for hybridization. The latent vectors (Z_1 and Z_2) obtained from principal component analysis (PCA). The important characters responsible for genetic divergence in the axis of differentiation in vector I (Z_1) were siliqua per plant (0.1487), siliqua length (0.1598) and seed yield per plant (0.1108) whereas, in vector II (Z_2) were 50% flowering (0.1901), plant height (0.1659), number of secondary branches per plant (1.1125) (Table 6). The role of days to 50% flowering, plant height and number of secondary branches in both the vectors were important components for genetic divergence in these materials. Islam and Islam (2000) reported days to 50% flowering, plant height, primary branches per plant and number of siliquae per plant contribute maximum in divergence in rapeseed and mustard. Begum et al. (2007) reported that branches per plant and number of number of seeds siliquae contributed the maximum towards divergence in the existing linseed germplasm.

Table 6. Relative contributions of the ten characters of 62 genotypes of *Brassica napus* L. to the total divergence

Characters	Principal Component	
	Vector-1	Vector-2
Days to 50% flowering	0.1256	0.1901
Days to maturity	-0.1310	-0.1407
Plant height (cm)	0.0643	0.1659
Primary branches per plant	-0.1931	-0.6343
Secondary branches per plant	0.1770	1.1125
Siliqua per plant	0.1487	-0.0443
Siliquae length (cm)	0.1598	-0.1166
Seeds per siliqua	-0.0725	-0.0539
1000-seed weight (g)	-0.1634	-0.6464
Seed yield per plant (g)	0.1108	-0.1884

CONCLUSION

It can be concluded that the hybridization between genotypes from cluster I with cluster IV might produce high level of segregating population. The crosses between the genotypes of cluster III with cluster V, cluster I with cluster IV, might produce high heterosis in respect of earliness and yield. Hence, the genotypes of these clusters could be used as parents in future breeding program for the improved variety of *Brassica napus* L.

ACKNOWLEDGMENTS

The authors are thankful to Ministry of Science and Technology Government of the People's Republic of Bangladesh for financial support through NST Fellowship.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

BBS. (2019). (Bangladesh Bureau of Statistics). Statistical Yearbook of Bangladesh. Bangladesh Bureau of Statistics, Ministry Planning, Government of Peoples Republic of Bangladesh, Dhaka. Pp.121

Begum, H., Alam, A.K.M.M., Chowdhury, M.J.A. and Hossain, M.I. (2007). Genetic divergence in linseed (*Linun usitatissimum* L.). *International Journal of Sustainable Crop Production*, 2(1): 4-6.

Bangladesh Economic Review. (2019). Economic Division, Ministry of Finance. Pp. 83

Bilal, M., Khan, S.A., Raza, H., Ali, F., Masaud, S., Khan, N.A., Hussain, I. and Khan, J. (2015). Evaluation of some indigenous rapeseed genotypes for adaptability and yield traits in the agro-climatic conditions of Mansehra. *International Journal of Biosciences,* **7**(5): 127-135.

FAO. (2019). Food and Agriculture Organization of United Nation.

Islam, M.S. and Islam, M.O. (2000). Genetic diversity in rapeseed and mustard (*Brassica* sp.). *Bangladesh Journal Plant Breeding and Genetics,* 13 (2): 25-30.

O'Brien, Richard D. (2008). fats and oils formulating and processing for applications (3rd). CRC Press. ISBN 9781420061666. Retrieved 18 April 2020. Pp. 37.

Pandey, R., Kumar, B. and Kumar, M. (2013). Genetic divergence for quantitative traits in indian mustard (*Brassica juncea* L. Czern & Coss). *American-Eurasian Journal of Agriculture and Environmental Science*, 13(3): 348-351.

Rameeh, V. (2013). Multivariate analysis of some important quantitative traits in rapeseed (*Brassica napus* L.) advanced lines. *Journal of Oilseed Brassica*, 4(2): 75-82.

Rameeh, V. (2015). Heritability, genetic variability and correlation analysis of some important agronomic traits in rapeseed advanced lines. *Cercetari Agronomice in Moldova*, 48(4): 71-80.

Rao, C.R. (1952). Advanced Statistical Methods in Biometrical Research. Pp. 45-110.

Sharafi, Y., Majidi, M.M., Jafarzadeh, M., and Mirlohi, A. (2015). Multivariate analysis of genetic variation in winter rapeseed (*Brassica napus* L.) cultivars. *Journal of Agricultural Science and Technology*, 17(5): 1319-1331.

Singh, P. (1983). Studies on genetic variability and diversity of rice. *Madras Agricultural Journal*, 70(7): 436-440.

Singh, R.K. and Chaudhary, B.D. (1985). Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi, India.

USDA (2002). Agricultural Statistics 2002. United States Department of Agriculture. ISBN 0160511135. Retrieved 20 March 2019.

Zaman, M.A., Khatun, M.T., Ullah, M.Z., Moniruzzamn, M. and Rahman, M.Z. (2010). Multivariate analysis of divergence in advanced lines of mustard (*Brassica spp.*). *Bangladesh Journal of Plant Breeding and Genetics,* 23(2): 29-34.